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Synthesis, Characterization and Antifungal Activity of Some New 5-Substituted 1,3,4-oxadiazole-2-thiols

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A series of 5-substituted 1,3,4-oxadiazol-2-thiols (**3a-i**) was synthesized by refluxing variably substituted organic acids (**Ra-i**) with methanol to the corresponding esters (**1a-i**). These esters were then converted to hydrazides (**2a-i**) by reaction with hydrazine hydrate in the presence of absolute ethanol which was followed by reaction with carbon disulfide and potassium hydroxide. Structure of the synthesized compounds was established by physicochemical and spectral data analysis. Synthesized compounds were subjected to antifungal activity. Antifungal activity was performed against *Aspergillus flavus*, *Mucor species*, *Aspergillus niger* and *Aspergillus fumigates*, with test compounds at a concentration of 200 µg/mL. Terbinafine was used as the standard drug.

Key Words: Synthesis, 1,3,4-oxadiazole, Antifungal activity.

INTRODUCTION

Considerable evidence has been accumulated in the past few years concerning the efficiency of 1,3,4-oxadiazoles in antimalarial[1], anticonvulsant[2], antiinflammatory[3], antitubercular[4], antibacterial[5] and antifungal[6] activities. Several Mannich bases have been synthesized from 1,3,4-oxadiazole-2-thione derivatives using different amines and formaldehyde. Most of these Mannich bases have been found to possess antibacterial, antifungal and antimicrobial activity[7]. Chelating properties of 1,3,4-oxadiazoles have been investigated using nickel(II), copper(II) and zinc(II) metals. Studies show that oxadiazoles have a better option to act as fungicides after chelating with metal ion[8]. 1,3,4-Oxadiazole-2-thiols are of great importance[9]. The presence of 2-thiol group on 1,3,4-oxadiazole ring enhances various biological activities. During the last decades, a number of derivatives of oxadiazoles have been synthesized and tested for their biological activity[10-13].

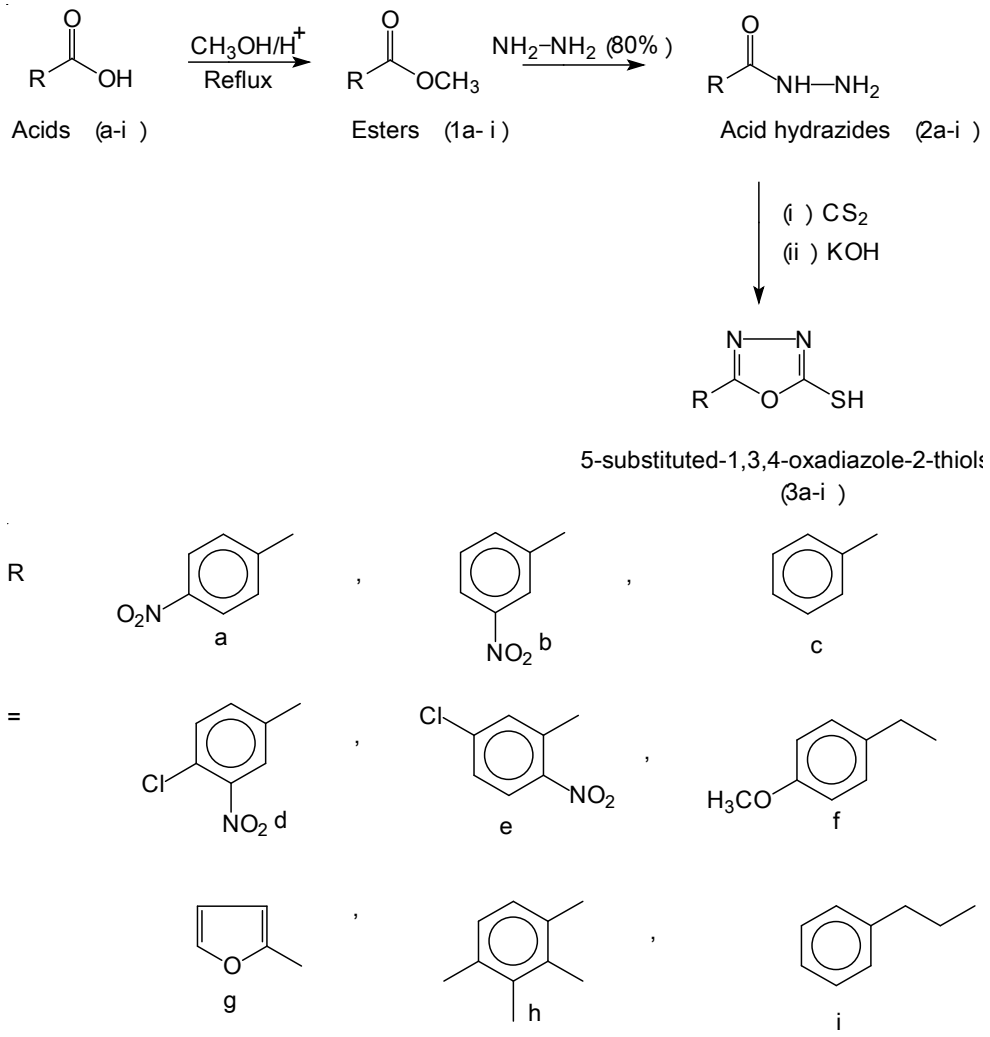
In view of the immense biological importance of 1,3,4-oxadiazoles and their 2-thiol derivatives, the present work was undertaken to design, synthesize and investigate the *in vitro* antifungal activity of some novel 5-substituted 1,3,4-oxadiazile-2-thiols (**3a-i**) (**Scheme-I**).

EXPERIMENTAL

Melting points of the synthesized compounds were recorded on Gallenkamp digital melting point apparatus MFB 595-101 M in open-end capillary tubes and were uncorrected. Thin layer chromatography was carried out on pre-coated silica gel plates (0.2 mm, E. Merck, 20 cm × 20 cm, $60F_{254}$). FTIR spectral data were recorded on Bio-Red Merlin spectrophotometer using KBr discs. ^{1}H NMR (300 MHz) and ^{13}C NMR (75.43 MHz) spectra were recorded on Bruker AM-250 spectrometer in DMSO and $CDCl_3$ solutions using TMS as internal standard. EIMS was recorded on Agilent VG: 70 SE mass spectrometer.

Synthetic method

Synthesis of methyl-4-nitrobenzoate (1a): 4-Nitrobenzoic acid (5 g, 0.029 mol) was taken in a 250 mL round bottom flask fitted with a reflux condenser and a calcium chloride guard tube. Absolute methyl alcohol (25 mL) and few drops of concentrated sulphuric acid were added and the reaction mixture was subjected to reflux for 4 h. The reflux time was monitored through TLC technique (silica; ethyl acetate: pet. ether, 1:3). After the completion of the reaction the excess of alcohol was distilled off on rotary evaporator. The residue was poured into 250 mL of water present in a separating funnel. Dichloromethane (20 mL) was added to the separating funnel and the mixture was stirred vigorously. The solution was allowed to stand and the methyl-4-nitrobenzoate in the dichloromethane separated and settled at the bottom of the separating funnel. The lower layer was carefully separated and the upper aqueous layer was rejected. The methyl-4-nitrobenzoate was returned to the separating funnel and shaken



Scheme-I

with a strong solution of sodium bicarbonate till all the free acid was removed. Methyl-4-nitrobenzoate was washed once with water and dried by pouring into a small dry conical flask containing 2 g of anhydrous magnesium sulphate. It was shaken for 5 min and allowed to stand for 1 h. The methyl-4-nitrobenzoate solution was filtered through a small fluted filter paper into a distillation flask. The flask was fitted with 360º thermometer, a condenser and a receiving flask. Dichloromethane was distilled off at 40 ºC and the solid methyl-4-nitrobenzoate was obtained from the flask. Ethanol was used as solvent for the recrystallization of the ester. Other esters (**1b-i**) were synthesized in the same way and their molar ratio and physical data are tabulated in Table-1.

Synthesis of 4-nitrobenzoic acid hydrazide (2a): Methyl-4-nitrobenzoate ester (7 g, 0.041 mol) was dissolved in absolute ethanol (40 mL) and taken in a flask fitted with a reflux condenser and a calcium chloride guard tube. Hydrazine hydrate (80 %, 13 mL) was added and the reaction mixture was subjected to reflux for *ca.* 8 h. The reflux time was monitored through TLC technique (silica; ethyl acetate:pet. ether, 1:2). After the completion of the reaction, the excess hydrazine was distilled off. The crude solid was collected, washed with water and recrystallized from 30 % aqueous ethanol. Other acid hydrazides (**2b-i**) were synthesized in the same way. The exact molar ratios and physical data of acid hydrazides (**2b-i**) are tabulated in Table-2.

Synthesis of 5-(4-nitrophenyl)-1,3,4-oxadiazole-2-thiol (3a): 4-Nitrobenzoic hydrazide (7 g, 0.038 mol) was dissolved in absolute ethanol in a 250 mL flask. Carbon disulfide (2 mL, 0.034 mol) was then added to the solution followed by the addition of potassium hydroxide (1.2 g, 0.019 mol) solution in 20 mL of water. The reaction mixture was thoroughly stirred and subjected to reflux. It was initially yellow which turned to green and then light yellow with the progress of the reaction. In each case the reaction time was monitored through TLC technique (silica; ethyl acetate:pet. ether, 1:2). Evolution of hydrogen sulfide gas was observed during each reaction. After completion of the reaction, excess of ethanol was removed under reduced pressure. The mixture was diluted with 200 mL of distilled water and acidified with 4 N hydrochloric acid to pH 2-3. It was then filtered, washed with diethyl ether and recrysallized from ethanol. Same synthetic procedure was also adopted for the synthesis of compounds (**3b-i**). The purification of synthesized compounds was achieved by recrystallization and purity of each compound was monitored by thin layer (TLC) and gas (GC) chromatography. Molar ratios and physical data of all 5-substituted-1,3,4-oxadiazole-2-thiols (**3a-i**) are given in Table-3.

TABLE-1
MOLAR RATIO AND PHYSICAL DATA OF ESTERS (**1a-i**)

Compound	R	Substituted acid (mol)	CH_3OH	Yield (%)	m.f.	m.w.	m.p. (ºC)
1a	4-Nitrophenyl	0.029	0.625	91	$C_7H_5O_4N$	167	96-97
1b	3-Nitrophenyl	0.029	0.625	92	$C_7H_5O_4N$	167	79-80
1c	Phenyl	0.057	0.750	94	$C_7H_6O_2$	122	198-200
1d	4-Chloro-3-nitrophenyl	0.049	1.000	90	$C_7H_4O_4NCl$	201	38-40
1e	5-Chloro-2-nitrophenyl	0.049	1.000	88	$C_7H_4O_4NCl$	201	Oil
1f	4-Methoxybenzyl	0.042	0.750	91	$C_9H_{10}O_3$	166	Oil
1g	2-Furoyl	0.089	1.000	93	$C_5H_4O_3$	112	Oil
1h	2-Phenethyl	0.066	1.000	91	$C_9H_{10}O_2$	150	Oil
1i	3,4,5-Trimethoxy-phenyl	0.023	0.625	90	$C_9H_{11}O_3$	212	Oil

TABLE-2
MOLAR RATIO ND PHYSICAL DATA OF ACID HYDRAZIDES (**2a-i**)

Compound	R	Substituted acid (mol)	NH_2NH_2 (mol)	Yield (%)	m.f.	m.w.	m.p. (ºC)
1a	4-Nitrophenyl	0.041	0.311	85	$C_7H_7O_3N_3$	181	216-218
1b	3-Nitrophenyl	0.059	0.413	84	$C_7H_7O_3N_3$	181	149-151
1c	Phenyl	0.065	0.331	86	$C_7H_8N_2O$	136	122-124
1d	4-Chloro-3-nitrophenyl	0.024	0.248	82	$C_7H_6O_3N_3Cl$	215	152-154
1e	5-Chloro-2-nitrophenyl	0.049	0.413	81	$C_7H_6O_3N_3Cl$	215	Oil
1f	4-Methoxybenzyl	0.030	0.311	85	$C_9H_{12}O_2N_2$	180	Oil
1g	2-Furoyl	0.062	0.311	83	$C_5H_6O_2N_2$	126	Oil
1h	2-Phenethyl	0.066	0.370	82	$C_9H_{12}N_2O$	164	160-161
1i	3,4,5-Trimethoxy-phenyl	0.037	0.331	83	$C_{10}H_{14}O_4N_2$	226	145-147

TABLE-3
MOLAR RATIOS AND PHYSICAL DATA OF 5-SUBSTITUED-1,3,4-OXAIDAZOLE-2-THIOLS (**3a-i**)

Compound	Substituted acid hydrazides (mol)	Carbon disulfide (mol)	Potassium hydroxide (mol)	Yield (%)	m.f.	m.w.	m.p. (ºC)
3a	0.038	0.034	0.019	75	$C_8H_5O_3N_3S$	223	196-198
3b	0.027	0.026	0.016	80	$C_8H_5O_3N_3S$	223	158-160
3c	0.058	0.046	0.016	78	$C_8H_6ON_2S$	178	201-203
3d	0.027	0.028	0.017	80	$C_8H_4O_3N_3SCl$	257	182-184
3e	0.018	0.023	0.012	79	$C_8H_4O_3N_3SCl$	257	122-124
3f	0.038	0.034	0.021	80	$C_{10}H_{10}O_2N_2S$	222	126-128
3g	0.039	0.022	0.011	80	$C_6H_4O_2N_2S$	168	118-120
3h	0.024	0.025	0.014	82	$C_{10}H_{10}ON_2S$	206	155-157
3i	0.026	0.029	0.021	80	$C_{11}H_{12}O_4N_2S$	268	160-162

RESULTS AND DISCUSSION

A series of 5-substituted 1,3,4-oxadiazol 2-thiols (**3a-i**) was synthesized. The synthesized compounds were characterized by physicochemical parameters and FTIR, ^{1}H NMR, ^{13}C NMR and mass spectral data. All the synthesized compounds showed characteristic bands in FTIR and NMR spectra. Expected molecular ion peaks ($M^+ + 1$) were observed for the entire compounds in mass spectra.

5-(4-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (3a): IR (KBr, ν_{max}, cm^{-1}): 2570 (S-H), 1580 (C=C), 1538 (C=N), 1009 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.10-8.12 and 8.37-8.38 (dd, 4H, Ar H), 15.0 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 128.02, 127.91, 125.04, 149.59, 125.05 and 127.91 (Ar C) 159.42 and 178.21 (oxadiazole moiety). MS (m/z) 223 (100 %), (M^+), 101 (12 %) (loss of nitrobenzene fragment), 177 (22 %) (loss of nitro group) 133 (24 %) loss of carbon monosulfide).

5-(3-Nitrophenyl)-1,3,4-oxadiazole-2-thiol (3b): IR (KBr, ν_{max}, cm^{-1}): 2569 (S-H), 1550 (C=C), 1530 (C=N), 1200 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 8.02-8 (d, 1H, Ar H), 7.28 (q, 1H, Ar H), 8.42 (d, 1H, Ar H), 8.66 (s, 1H, Ar H), 12.35 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 129.04, 126.77, 150.23, 125.11, 126.04 and 126.77 (Ar C) 164.51 and 181.33 (oxadiazole moiety). MS (m/z) 223 (100 %), (M^+), 101 (12 %) (loss of nitrobenzene fragment), 177 (22 %) (loss of nitro group) 133 (24 %) loss of carbon monosulfide).

5-Phenyl-1,3,4-oxadiazole-2-thiol (3c): IR (KBr, ν_{max}, cm^{-1}): 2560 (S-H), 1554 (C=C), 1514 (C=N), 1108 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28(d, 1H, Ar H), 7.24-7.26 (q, 1H, Ar H), 7.21 (m, 1H, Ar H), 7.30 (q, 1H, Ar H), 12.33 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 127.11, 126.21, 125.02, 125.11, 125.02 and 126.21 (Ar C) 158.66 and 175.72 (oxadiazole moiety). MS (m/z) 178 (100 %), (M^+), 101 (12 %) (loss of nitrobenzene fragment), 177 (22 %) (loss of nitro group) 133 (28 %) loss of carbon monosulfide).

5-(4-Chloro-3-nitrophenyl)-1,3,4-oxadiazole-2-thiol (3d): IR (KBr, ν_{max}, cm^{-1}): 2554 (S-H), 1510 (C=C), 1527 (C=N), 1025 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.35 (dd, 1H, Ar H), 8.68 (d, 1H, Ar H), 8.70 (s, 1H, Ar H), 14.82 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 128.41, 127.21, 150.42, 130.21, 129.71 and 126.33 (Ar C) 160.02 and 180.20 (oxadiazole moiety). MS (m/z) 257 (100 %), (M^+), 101 (12 %) (loss of nitrobenzene fragment), 211 (24 %) (loss of nitro group) 167 (26 %) loss of carbon monosulfide).

5-(5-Chloro-2-nitrophenyl)-1,3,4-oxadiazole-2-thiol (3e): IR (KBr, ν_{max}, cm^{-1}): 2562 (S-H), 1545 (C=C), 1518 (C=N), 1210 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.20 (d, 1H, Ar H), 8.40 (dd, 1H, Ar H), 8.38 (d, 1H, Ar H), 12.84 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 130.08, 151.66, 126.34, 125.61, 133.01, 128.81 (Ar C) 158.88 and 179.01 (oxadiazole moiety) MS (m/z) 223 (100 %), (M^+), 101 (14 %) (loss of nitrobenzene fragment), 211 (25 %) (loss of nitro group) 167 (26 %) loss of carbon monosulfide).

5-(4-Methoxybenzyl)-1,3,4-oxadiazole-2-thiol (3f): IR (KBr, ν_{max}, cm^{-1}): 2930 (C-H), 2523 (S-H), 1503 (C=C), 1535 (C=N), 1306 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28 (dd, 1H, Ar H), 7.89 (dd, 1H, Ar H), 7.89 (dd, 1H, Ar H), 7.28 (dd, 1H, Ar H), 3.45 (s, 3H, methoxyl H), 2.84 (s, 2H, methylene H),12.48 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 130.05, 127.62, 125.61, 136.21, 125.61 and 127.62 (Ar C) 42.21 (methylene C) 56.61 (methoxyl C) 162.44 and 177.64 (oxadiazole moiety). MS (m/z) 222 (100 %), (M^+), 101 (16 %) (loss of nitrobenzene fragment), 207 (22 %) (loss of nitro group) 163 (24 %) loss of carbon monosulfide).

5-(2-Furoyl)-1,3,4-oxadiazole-2-thiol (3g): IR (KBr, ν_{max}, cm^{-1}): 2533 (S-H), 1578 (C=C), 1520 (C=N), 1140 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28 (dd, 1H, Ar H), 7.21 (q, 1H, Ar H), 8.48 (d, 1H, Ar H),13.02 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 138.12, 130.16, 128.62 and 128.91 (Ar C) 160.28 and 175.77 (oxadiazole moiety). MS (m/z) 168 (100 %), (M^+), 101 (12 %) (loss of nitrobenzene fragment), 138 (25 %) loss of carbon monosulfide).

5-(2-Phenethyl)-1,3,4-oxadiazole-2-thiol (3h): IR (KBr, ν_{max}, cm^{-1}): 3010 (C-H), 2550 (S-H), 1567 (C=C), 1533 (C=N), 1022 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.28(dd, 1H, Ar H), 7.21 (m, 1H, Ar H), 7.25 (m, 1H, Ar H), 7.21 (m, 1H, Ar H), 3.20 (t, 4H, 2 methylene H),12.68 (s, 1SH); ^{13}C NMR (DMSO-d_6) δ (ppm): 129.01, 127.21, 125.41, 125.11, 125.41 and 127.21 (Ar C) 30.11, 35.21 (methylene C) 160.09 and 179.06 (oxadiazole moiety). MS (m/z) 206 (100 %), (M^+), 101 (14 %) (loss of nitrobenzene fragment), 162 (26 %) (loss of nitro group) 133 (24 %) loss of carbon monosulfide).

5-(3,4,5-Trimethoxyphenyl)-1,3,4-oxadiazole-2-thiol (3i): IR (KBr, ν_{max}, cm^{-1}): 2990 (C-H), 2526 (S-H), 1540 (C=C), 1510 (C=N), 1030 (C-O); ^{1}H NMR (DMSO-d_6) δ (ppm): 7.25 (s, 1H, Ar H), 7.25 (s, 1H, Ar H), 3.40 (s, 9H, 3 methoxyl H), 15.0 (s, 1H); ^{13}C NMR (DMSO-d_6) δ (ppm): 129.66, 126.01, 139.01, 139.66, 139.01 and 126.01 (Ar C) 54.33 (methoxcyl C) 158.32 and 176.44 (oxadiazole moiety). MS (m/z) 268 (100 %), (M^+), 101 (16 %) (loss of nitrobenzene fragment), 233 (22 %) (loss of nitro group) 179 (24 %) loss of carbon monosulfide).

Antifungal activity: The synthesized 5-substituted 1,3,4-oxadiazole-2-thiols (**3a-i**) were tested by agar tube dilution method[14,15], for their *in vitro* fungicidal bioassay. All experiments were done in three replicates. Following four fungal strains *Aspergillus flavus*, *Mucor species*, *Aspergillus niger* and *Aspergillus fumigatus* were used in antifungal assay. All fungal strains were grown on 6.5 % SDA (sabouraud dextrose agar, pH 5.7) at 28 ºC and preserved at 4 ºC in refrigerator. 100 mm slants with sterilized SDA were prepared by adding each compound at 200 mg/mL concentration. Terbinafine (200 µg/mL) was used as standard drug while DMSO was used as negative control. Each slant was inoculated with 4 mm piece of respective fungal strain and incubated at 28 ºC for 7-10 days. Linear growth inhibition results of 5-substitutd 1,3,4-oxadiazole-2-thiols (**3a-i**) are given in Table-4. The antifungal activity analysis showed only significant activity for the **3d**, **3f** and **3h** against *Mucor species*, *Aspergillus* and *Aspergillus fumigatus*, but do not exhibit significant activity against *Aspergillus flavus*. However, compound **3f** showed linear growth inhibition against *Mucor species* and *Aspergillus fumigatus* even more than the standard drug.

TABLE-4
ANTIFUNGAL ACTIVITY OF 5-SUBSTITUTED 1,3,4-OXADIAZOLE-2-THIOLS (**3a-i**)

Compound	Per cent linear growth inhibition 200 (µg/mL)			
	Aspergillus flavus	*Mucor species*	*Aspergillus niger*	*Aspergillus fumigates*
3a	48.02	62.11	53.00	59.05
3b	39.21	45.22	23.36	49.31
3c	33.36	50.25	46.17	17.43
3d	58.09	87.66	74.79	76.21
3e	47.09	55.43	35.65	60.08
3f	84.10	100.00	81.20	100.00
3g	21.24	47.71	25.27	13.15
3h	69.13	80.96	75.20	87.33
3i	65.13	72.04	59.33	42.07
Terbinafine	100.00	90.06	110.80	98.41

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